TRYPTOPHAN PYRROLASE ACTIVITY IN THE LIVER OF ADULT RANA PIPIENS [1]

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It has been shown by Knox and Mehler (1950) that the activity of mammalian liver tryptophan pyrrolase (peroxidase-oxidase) was greatly increased after injection of tryptophan. The enzyme has been found in the livers of a variety of mammals but not in other tissues (Knox, 1955). Knox and Mehler (1951) further demonstrated that this induction of increased enzyme activity was not controlled by substrate alone. Increased liver enzyme levels could be produced by various stressing conditions, such as section of the spinal cord (Thompson and Mikuta, 1954), and by injections of such substances as histidine and tyrosine (Knox and Mehler, 1951) and histamine and adrenalin (Knox, 1951) in intact animals. In adrenalectomized animals liver enzyme activity could be increased by injections of adrenocorticotropic hormone (Geschwind and Li, 1953) and the glucocorticoid hormones (Thompson and Mikuta, 1954; Knox and Auerbach, 1955). Knox and Auerbach (1955) concluded that there were probably two types of inducing agents, the substrate and an adrenal hormone, and that the enzyme could be induced in adrenalectomized rats by the substrate independently of the hormone. Civen and Knox (1959) further demonstrated that the hormonal induction was independent of substrate concentration and not due to an increase in the level of tryptophan in the tissues produced by the glucocorticoid tissues. More recently Feigelson and Greengard (1961) have demonstrated that rat liver tryptophan pyrrolase is activated by an iron protoporphyrin located in the microsomes.

In the course of investigations on the synthesis of tryptophan pyrrolase during the embryonic development (to be reported elsewhere) of *Rana pipiens* it was found that the controlling mechanisms of tryptophan pyrrolase activity in the frog livers are quite different from those of the adult mammal. It is the results of this investigation on the liver of adult *Rana pipiens* that are reported in the present paper.

Materials and Methods

Large adult male and female *Rana pipiens* were obtained from Vermont and maintained in running tap water at 12–18° C. The procedure for assay of the enzyme was that described by Knox and Auerbach (1955) with two exceptions. The homogenate was prepared by grinding the liver in either a Teflon-Pyrex homogenizer [2] or a Virtis "23" homogenizer [3] for 2–3 minutes, and secondly the reactions were run in an air atmosphere. All other details were *exactly* as described by Knox and Auerbach. Under the conditions specified by these authors

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[2] Kontes Glass Co., Vineland, New Jersey.

[3] Virtis Company, Inc., Yonkers, New York.

enzyme activity was directly proportional to concentration of frog liver homogenate. The Knox and Auerbach procedure is based on the conversion of tryptophan to kynurenine which is the product actually measured during the assay. This conversion, however, is a two-step reaction, tryptophan pyrrolase catalyzing the first step, the conversion of tryptophan to formylkynurenine. The enzyme formylase, which catalyzes the conversion of formylkynurenine to kynurenine, the second step, is found in excess in mammalian liver homogenates and is not rate-limiting (Knox and Mehler, 1950). In all control and experimental frog livers, no reaction products absorbing at 321 mμ were detected. This indicated the absence of formylkynurenine, and formylase, therefore, is probably present in excess in frog livers and is not rate-limiting. L-tryptophan, L-histidine (free base), bovine adrenocorticotropic hormone (ACTH), cortisone acetate, and hydrocortisone acetate were obtained from the Nutritional Biochemical Laboratory, Cleveland, Ohio. Pituitaries were obtained by dissection from large adult females. All injections were given intraperitoneally. All enzyme activities are expressed in terms of μ moles of kynurenine formed per hour at 38° C., per gram dry weight of liver, ± standard error of the mean.

Results

During the early stages of this investigation adult females were used exclusively since it was our intention first to study the activity of the enzyme, under various

Table I

Basal activity of enzyme in liver of adult Rana pipiens females (non-ovulated) during the breeding season

Date	Number of animals	Activity*
4/27/60–5/3/60	10	11.59 ± 1.99
5/4/60–5/11/60	21	19.68 ± 2.20
5/18/60–5/23/60	9	25.60 + 2.62

* μ moles kynurenine formed per hour at 38° C., per gram dry weight of liver, ± standard error of the mean.

conditions, in the adult and ultimately to relate these findings to a study of the enzyme during embryonic development, starting with the ovarian egg. The results at first glance seemed to indicate that the enzyme activity in the female was extremely variable and that the frog was a rather poor experimental animal for this type of study. A more detailed analysis revealed that basal activity of the enzyme in the female was indeed variable but that the extreme variability was due to a progressive increase in enzyme activity following hibernation and during the breeding season. Table I summarizes these findings. It will be noted that a more than 100% increase in enzyme activity (11.59 ± 1.99 to 25.60 ± 2.62 μ moles kynurenine/hour/gm. dry weight of liver) occurred, in non-ovulated females, over an approximately 4-week period following hibernation.

A comparison of liver enzyme activity in females which had ovulated in nature with non-ovulated females revealed that sometime during or following the period of ovulation, enzyme activity radically decreased from a value of 25.10 ± 2.12 to 8.80 ± 1.00 (Table II).

It seemed probable that perhaps pituitary hormones were changing in activity

TABLE II

Basal activity of enzyme in liver of adult Rana pipiens ovulated females vs. non-ovulated females

State of ovulation	Number of animals	Activity*
Ovulated	17	8.80 ± 1.00
Non-ovulated	9	25.10 ± 2.12

* μ moles kynurenine formed per hour at 38° C., per gram dry weight of liver, ± standard error of the mean.

during the breeding season and affecting the enzyme activity. Accordingly pituitaries isolated from mature females were injected into mature ovulated and non-ovulated females. The results are summarized in Table III. In ovulated females the control activities were of the low pre-breeding season values. Injection of pituitary lowered the enzyme activity to 4.45 ± 0.161. That this activity-lowering effect of pituitary injection was not due to events either preceding or occurring simultaneously with ovulation is shown by the results of pituitary injections on non-ovulated females (Table III). In this case the non-ovulated females, after injection of pituitaries, ovulated, but the liver enzyme activity remained at a high level. Further consideration will be given to this point in the discussion section of this paper.

Since the results indicated that enzyme activity in the female varied to a considerable extent during the breeding season, subsequent experiments were carried out on the male frog. Comparable suppression of enzyme activity following pituitary injections was also encountered in the male. In fact, enzyme activity in the male was almost identical with that of the female after this treatment, 4.99 ± 0.619 vs. 4.45 ± 0.161 in the female (Table III). It was interesting to note that basal activity in the male, 9.64 ± 1.26 (Table IV), very closely approximated the basal activity in female livers after ovulation (8.80 ± 1.00, Table II).

Table IV summarizes liver enzyme activity in the male frog after injections of various compounds known to increase activity in the normal intact mammal.

TABLE III

Activity of enzyme in liver of adult Rana pipiens after pituitary injections

Number, sex, and state of ovulation of animals	Treatment	Time (hrs.) assayed after injection	Activity*
14, females, ovulated	2–4 pituitaries in 1 ml. of 10% Holtfreter's solution	5	4.45 ± 0.161
6, females, ovulated	1 ml. of 10% Holtfreter's solution	5	7.41 ± 1.18
6, females, non-ovulated before injection	2 pituitaries in 1 ml. of 10% Holtfreter's solution	18	24.16 ± 2.53
6, females, non-ovulated before injection	1 ml. of 10% Holtfreter's solution	18	22.36 ± 1.79
6, males	5 pituitaries in 1 ml. of 10% Holtfreter's solution	$14\frac{2}{3}$	4.99 ± 0.619
6, males	1 ml. of 10% Holtfreter's solution	$14\frac{2}{3}$	9.80 ± 0.41

* μ moles kynurenine formed per hour at 38° C., per gram dry weight of liver, ± standard error of the mean.

TABLE IV

Enzyme activity in liver of adult male Rana pipiens after various treatments

Number of animals	Treatment	Activity*
12	2 ml. L-histidine**	6.44 ± 0.66
12	2 ml. 0.65% NaCl	9.71 ± 0.97
6	ACTH***	7.56 ± 2.11
6	15% gelatin in 0.5% phenol****	9.65 ± 1.21
8	0.5 ml. cortisone acetate†	9.34 ± 1.00
8	0.5 ml. 0.65% NaCl††	9.59 ± 0.73
6	1 ml. hydrocortisone acetate†††	9.20 ± 0.98
6	1 ml. 0.65% NaCl	9.81 ± 1.47
6	3 ml. L-tryptophan††††	40.50 ± 4.90
6	3 ml. 0.65% NaCl	9.49 ± 0.53

* Micro moles kynurenine formed per hour at 38° C., per gram dry weight of liver, ± standard error of the mean. All animals sacrificed and livers assayed 4–6 hours after the last injection.

** Six hundred and thirty mg. in 12 ml. of 0.65% NaCl.

*** One-quarter ml. (8 units/ml. of 15% gelatin in 0.5% phenol); 0.1 ml. 24 hours later; then 0.25 ml. 16 hours later.

**** One-quarter ml.; 0.1 ml. 24 hours later; then 0.25 ml. 16 hours later.

† Ten mg./4 ml. of 0.65% NaCl; for 3 days at 24-hour intervals.

†† For 3 days at 24-hour intervals.

††† Eighty mg./10 ml. 0.65% NaCl.

†††† Seventy-five mg./15 ml. of 0.65% NaCl.

Injections of L-tryptophan produced a 427% increase in enzyme activity. Rather surprisingly, injection of the adrenal cortical hormones, cortisone acetate and hydrocortisone acetate, produced no significant change in enzyme activity in contrast to the increases in activity found in the mammal after their injection. Similarly, L-histidine and ACTH produced no increase as they do when injected into the mammal, and in fact the results suggest that these substances may produce an inhibition instead of an augmentation.

DISCUSSION

The observation that liver tryptophan pyrrolase activity increases in the female frog during the breeding season, to our knowledge, has no counterpart in the recorded observations of activity in the female mammal. Since no investigation, to our knowledge, has been carried out in the female mammal to check tryptophan pyrrolase activity during the estrous cycle it would be presumptuous to state that the amphibian, as exemplified by the frog, is in direct contrast to the mammal with respect to this observation. It is possible that a comparable investigation carried out in the mammal would lead to similar results. The mechanism of this increase remains to be elucidated.

It is, however, well known that in female frogs maintained at temperatures above 15–18° C. for several weeks there is a rapid consumption of the fat bodies and even of the eggs within the ovaries (Rugh, 1935). If female frogs are maintained at 20–25° C. for prolonged periods, abnormal eggs are produced after ovulation. It is possible that the ovarian eggs and fat bodies of female frogs which have not ovulated early in the breeding season begin to degenerate at the relatively warmer temperatures prevalent during the latter part of the season.

Concomitant with this degeneration one could assume a release of tryptophan (from the fat bodies and eggs into the maternal circulation) which is eventually transported to the liver where a tryptophan induction of enzyme activity would occur. While no figures are available, it is our observation that towards the end of the breeding season the percentage of non-ovulated females furnishing 95–100% fertilizable and normally developing eggs diminishes sharply. The return to relatively low enzyme activity following ovulation would offer partial support to this hypothesis. The failure of pituitary injections in non-ovulated females (although bringing about ovulation in these animals) to lower the enzyme activity would require under this hypothesis that the blood tryptophan concentration was sufficiently high to maintain appreciable enzyme activity (or continued induction) for some time after ovulation had occurred. Two experiments carried out in this laboratory indicate that one week after ovulation had been induced by pituitary injection, and the females stripped, the enzyme activity had dropped to its pre-breeding season value. Enzyme activity for these experiments was 8.90 μ moles of kynurenine per hour, per gm. dry weight of liver. Measurements of ovarian and fat body vs. blood tryptophan concentrations during the breeding season would be of value in testing the validity of this hypothesis.

It has been demonstrated by Sims and Bishop (1947) that the pituitary gland of *Rana pipiens* does undergo seasonal variations, increasing in gonadotropic potency between January and March. The possibility that pituitary hormones are increasing during the breeding season and affecting the adrenal cortical hormone balance, which in turn brings about an increase in enzyme activity, seems to be negated by the decrease in activity following pituitary injection. It is implicit in the hypothesis that the addition of pituitary should either augment enzyme activity or have no effect if the synthesis of enzyme is going on at a maximum rate before injection of pituitary. The most probable explanation for the pituitary effect is that the decrease of activity is due to growth hormone, for it has been demonstrated by Wood and Knox (1954) that growth hormone when injected into the mouse brings about a decrease in liver enzyme activity. Estrogenic hormones undoubtedly vary in concentration during the breeding season and should be tested for their effect on enzyme activity.

A third explanation for the increase in enzyme activity during the breeding season remains to be considered. It is possible that the feeding habits of the adult female are extremely variable and it is conceivable that the female upon breaking hibernation consumes a diet rich in tryptophan, leading to an induction of enzyme activity. Following ovulation the consuming of a diet low in tryptophan would in turn lead to a decrease in enzyme activity. A careful search of the literature has revealed no information as to the variation in the food habits of the female frog during this period of the year.

Recently Feigelson and Greengard (1961) have demonstrated that rat liver tryptophan pyrrolase is activated by an iron protoporphyrin present in liver microsomes. It has been further demonstrated (Greengard and Feigelson, 1961) that tryptophan treatment caused an approximately 20% increase in the iron protoporphyrin concentration of liver microsomes, and that the initial rise in enzyme activity after tryptophan treatment was due to an increased degree of saturation of the enzyme with respect to the activator. It is possible that the increases

noted in the enzyme activity of frog liver homogenates during the breeding season and following tryptophan administration and the suppression of activity following pituitary injection are due to changes in the iron protoporphyrin content of liver microsomes. Experiments carried out on the frog which are comparable to those of Feigelson and Greengard will elucidate the role of this activator under the conditions mentioned above.

The failure of the adrenal cortical hormones and of ACTH and L-histidine to produce an augmentation of enzyme activity is in direct contrast to the increases in activity found in the mammal after similar treatment (Knox and Mehler, 1951; Geschwind and Li, 1953). The data for ACTH and L-histidine suggest that these substances may in fact bring about a decrease in activity but further experiments are needed to substantiate this possibility. Although Carstensen *et al.* (1961) have demonstrated the production of aldosterone and corticosterone by *Rana catesbiana* adrenal tissue under the stimulation of bovine ACTH, there appears to be no published information regarding the adrenal cortical hormones in *Rana pipiens,* and it is possible that the amphibian cortical hormones are quite different from those of the mammal. Injections of frog adrenal homogenates would be extremely helpful in establishing this hypothesis.

The frog appears to be an ideal organism for studying the mechanism of substrate induction in metazoa of this enzyme, for the results are not complicated by the possibility of an effect being mediated through the adrenal.

Summary

1. The effect of the injection of L-tryptophan, L-histidine, ACTH, cortisone acetate, hydrocortisone acetate, and pituitary on liver tryptophan pyrrolase activity was studied in adult male and female *Rana pipiens.*

2. L-tryptophan produced a 427% increase in activity but cortisone acetate and hydrocortisone acetate injections had no effect. ACTH and L-histidine injections produced a slight decrease in activity. Pituitary injections brought about a marked decrease in activity.

3. In adult females there was a progressive increase in enzyme activity following hibernation and during the breeding season. This progressive increase was followed by a sharp drop in activity following ovulation.

4. The results are discussed in terms of the mammalian data.

LITERATURE CITED

Carstensen, H., A. C. J. Burgers and C. H. Li, 1961. Demonstration of aldosterone and corticosterone as the principal steroids formed in incubates of adrenals of the American bullfrog (*Rana catesbiana*) and stimulation of their production by mammalian adrenocorticotropin. *Gen. Comp. Endocrinol.,* 1: 37–50.

Civen, M., and W. E. Knox, 1959. The independence of hydrocortisone and tryptophan inductions of tryptophan pyrrolase. *J. Biol. Chem.,* 234: 1787–1790.

Feigelson, P., and O. Greengard, 1961. A microsomal iron-porphyrin activator of rat liver tryptophan pyrrolase. *J. Biol. Chem.,* 236: 153–157.

Geschwind, I. I., and C. H. Li, 1953. Influence of hypophysectomy and of adrenocorticotropic hormone on a mammalian adaptive enzyme system. *Nature,* 172: 732–733.

Greengard, O., and P. Feigelson, 1961. The activation of rat liver tryptophan pyrrolase *in vivo* by its substrate. *J. Biol. Chem.,* 236: 158–161.

KNOX, W. E., 1951. Two mechanisms which increase *in vivo* the liver tryptophan peroxidase activity: specific enzyme adaptation and stimulation of the pituitary adrenal system. *Brit. J. Exp. Pathol.,* **32**: 462–469.

KNOX, W. E., 1955. Tryptophan oxidation. *In:* Methods in Enzymology, edited by S. P. Colowick and N. O. Kaplan, Academic Press Inc., Publishers, New York, N. Y., pages 242–253.

KNOX, W. E., AND V. H. AUERBACH, 1955. The hormonal control of tryptophan peroxidase in the rat. *J. Biol. Chem.,* **214**: 307–313.

KNOX, W. E., AND A. H. MEHLER, 1950. The conversion of tryptophan to kynurenine in liver. I. The coupled tryptophan peroxidase system forming formylkynurenine. *J. Biol. Chem.,* **187**: 419–438.

KNOX, W. E., AND A. H. MEHLER, 1951. The adaptive increase of the tryptophan peroxidase-oxidase system of liver. *Science,* **113**: 237–238.

RUGH, R., 1935. Ovulation in the frog. I. Pituitary relations in induced ovulation. *J. Exp. Zool.,* **71**: 149–162.

SIMS, W. L., AND D. W. BISHOP, 1947. Seasonal variation in the gonadotropic potency of dried anterior pituitaries of *Rana pipiens. Science,* **106**: 588.

THOMPSON, J. F., AND E. T. MIKUTA, 1954. Effect of total body X-irradiation on the tryptophan peroxidase activity of rat liver. *Proc. Soc. Exp. Biol. Med.,* **85**: 29–32.

WOOD, J. S., AND W. E. KNOX, 1954. Studies on liver tryptophan peroxidase in mice bearing a transplantable sarcoma. *Proc. Amer. Assn. Cancer Research,* **1**: 52–53.